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Evaluation of the Acid Digestion Method with Different Solvent Combination for the Determination of Iron, Zinc and Lead in Canned Sardines

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Abstract : The objective of this paper was to evaluate the efficiency of the five different combinations of solvent used in the classical wet digestion procedure for the determination of Fe, Pb and Zn contents in canned sardine. The different solvent combinations evaluated were HNO_3, HNO_3: HCl (1:1), HNO_3: HCl (1:2), HNO_3: HCl (1:3) and HNO_3 : H_2SO_4 (1:1). The combination with the most reliable performance recovery was employed to study the level of Pb, Fe and Zn in canned sardines manufactured from different countries and with different shelf lives. Results indicated that this combination, HNO_3: HCl (1:2), produced the most satisfactory percentage recovery. The content of Fe, Pb and Zn in the canned sardines were found to be below the permitted level established in the Food Act 1983 and Food Regulation 1996. However, leaching of Fe and Pb from the can was likely to have occurred in products stored for a longer period of time. This was evidenced by the determination of metal contents in the gravy from the canned sardines. Nevertheless, leaching of Zn was not observed.

Abstrak : Efisiensi kaedah pencernaan berdasarkan lima kombinasi solven yang berlainan untuk penentuan kandungan Fe, Pb dan Zn dalam 'sardin dalam tin' dikaji. Kombinasi solven tersebut termasuk HNO_3, HNO_3: HCl (1:1), HNO_3: HCl (1:2), HNO_3: HCl (1:3) and HNO_3 : H_2SO_4 (1:1). Kombinasi dengan efisiensi yang paling memuaskan digunapakai untuk penentuan kandungan Fe, Pb dan Zn dalam sardin yang dihasilkan dari negara yang berlainan dan tempoh simpanan yang berbeza. Keputusan menunjukkan bahawa kandungan ketiga-tiga logam tersebut adalah di bawah paras yang dibenarkan oleh Akta Makanan 1983 dan Regulasi Makanan 1996. Walaubagaimanapun, sardin yang disimpan lebih lama didapati mengandungi Fe dan Pb yang lebih tinggi. Pemerhatian sedemikian tidak didapati pada kandungan Zn.

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Introduction

Canned foods offer a shortcut in meal preparation which is most favoured by those who are stretched for time. Canned fish, particularly canned sardines products manufactured either locally or imported, is very popular in supermarket chain, public markets and small grocery retail outlets. Owing to the great consumption of this product, the safety issues related to the possibility of heavy metals contamination are of concern. The subject of heavy metals is receiving increasing scrutiny in food industry due to increasing incidents of contamination in agriculture and seafood sources. Apart from the threat from polluted environment, canned food is subjected to heavy metal contamination during the canning process. Solder used in manufacture of cans has been recognized as a source of lead contamination during canning [1]. In light of that, routine monitoring is performed to ascertain the metal contents are within the range of the permitted levels. In addition, it is essential to identify the interaction between the foodstuff and its package, particularly when it is being purchased and consumed nationwide on a regular basis.

Sample digestion is often a necessary step before analysis of metal concentrations with highly sensitive spectroscopic techniques. Classically, it involves dissolution/ digestion of samples on the hotplate with different combinations of mineral acids such as HNO_3 + HCl, HNO_3 + H_2O_2, HNO_3 + HF, etc. In the past two decades, a drastic change had been made in the sample preparation procedures. The introduction of microwaves, with both open and closed pressurized systems, offers an alternative that has allowed a considerable reduction in the total time of analyses as well as the risk of sample contamination. Mester *et al.* revealed that the open wet digestion method is more time-consuming and complicated than the microwave methods without any advantage in terms of the digestion efficiency [2]. Motrenko *et al.* recommended the microwave assisted digestion procedure as this leads to more accurate results [3]. In spite of that, the conventional wet digestion procedure remains popular and important in most small-scale laboratories. The efficiency of the digestion method strongly influences the accuracy of the final results depending on the type of samples. For example, the nitric acid procedure was reported to be the most efficient in determination of Cd, Mn and Ni from compost samples. The sulphuric acid procedure however, yielded the lowest recovery of Pb [4]. Digestion procedure could be the source of uncertainty and contamination due to incomplete mineralization of the organic matter, formation of volatile compounds and atmospheric contamination. Therefore, it is crucial to evaluate the acid digestion protocol for an accurate determination of metal contents in the sample of interest.

For these reasons, this paper attempted to evaluate five acid digestion mixtures in the determination of iron, zinc and lead in canned sardines. Iron and zinc are essential element for the biological functions of human body. Iron is vital for transportation of oxygen and electrons and zinc is an important constituent for more than 100 enzymes. Lead however is a highly toxic element that is heavily used in can making industries. An alloy containing 98% lead/ 2% tin is usually used to solder the side seam of most of the tinplated cans [1]. The complex matrix with the best recovery efficiency was adopted to determine the Pb, Fe and Zn in the canned sardines with different shelf life and manufacturing country.

samples were pulverized and homogenized prior to the digestion procedure.

Digestion procedure

Open wet digestion with five different acid mixtures were evaluated: HNO_3; HNO_3 + HCl (1:1); HNO_3 + HCl (1:2); HNO_3 + HCl (1:3); HNO_3 + H_2SO_4 (1:1). The samples were digested in triplicates and were analyzed for Fe, Zn and Pb. Three quality assurance samples were prepared: the blank, the non-spiked samples and the spiked samples. For the digestion solution that favored formation of precipitate due to fatty residue, 2 ml of ethanol was added for digestion at room temperature.

Recoveries

For recovery analysis, samples were spiked with a known amount of metals. Approximately 1.00 g of samples was spiked with Fe and Zn standard solution to attain concentrations of 5 mg/kg and 10 mg/kg. The samples were spiked with Pb standard solution to acquire a concentration of 0.5 mg/kg and 1.0 mg/kg. The samples were mixed well and subjected to digestions with different acid mixtures. The digested samples were filtered, diluted to 50 ml and analyzed with FAAS. The percentage recovery of the samples was calculated as follows:

$$\% \text{ Recovery} = \frac{\textit{metals content of the spiked samples} - \textit{metals content of non-spiked samples}}{\textit{The expected metals content}} \times 100\%$$

Methodology

Apparatus

Flame atomic absorption spectrometry (FAAS) analyses were carried out by a FAAS Perkin Elmer Model with hollow cathode lamps.

Reagents

All standard solutions for AAS (1000mg/L) and acids were commercially available. The working standard solutions were prepared and stored in polyethylene container at 4°C. Deionised water was used for all dilution purposes.

Evaluation of the acid digestion methods

Samples

Canned sardines manufactured in Malaysia with a shelf life less than one year were used in the evaluation of the acid digestion methods. The fish canned was Sardinops sp. The cans were opened and the gravy drained. The sardines were rinsed with deionized water and freeze-dried. The freeze-dried

Determination of Fe, Zn and Pb is canned sardines

Thirty samples of canned sardines in tomato sauce representing two manufacturing countries and three categories of shelf life were analyzed for Pb, Fe and Zn. The shelf life concerned was less than 1 year, 1-2 years and 2-3 years. The manufacturing countries of the products were Malaysia and Thailand. The fish from the Malaysian and Thai products were *Sardinops* sp. and *Sardinellas* sp., respectively. The cans were opened and the gravy was drained and set aside for analysis. The sardines were rinsed with deionised water and freeze dried prior to digestion with HNO_3: HCl (1:2).

Approximately 5.00 g of finely powdered samples were digested with 5 ml of hydrochloric acid (37 % HCl) for 10 minutes. Five millilitres of nitric acid (69 % HNO_3) was later added slowly. The samples were swirled gently, covered with watch glass and left at room temperature until most of the samples were dissolved. The solutions were heated on the hot plate for 30 minutes until yellow fume was released. After cooling, the acid solutions were filtered into 50 ml volumetric flask marked up with deionized water. Samples were digested in triplicates and analyzed for Pb, Fe and Zn.

The drained gravy was homogenized with blender prior to digestion. Approximately 10 ml of sample was digested with 5 ml of nitric acid and 10 ml of hydrochloric acid. The sample was swirled gently, covered with watch glass and left at room temperature for about 1 hour until most of the sample was dissolved. Sample was then heated on the hotplate until a yellow fume was released and the solution became clear. After cooling, the acid solution was filtered into a 50 ml volumetric flask and marked up with deionised water. Three replications were prepared for each sample for the analysis of Pb, Fe and Zn.

Statistical Analysis

Statistical analysis was performed with One-way ANOVA to identify the significant differences in the metal contents due to different solvent combination.

fluctuated from 75 – 175 %. Application of HNO_3 alone was reported to be not as effective for determination of Zn and Fe in biscuit samples due to poor percentages recovery [5]. Krumgalz and Fainshtein studied the efficiency of HNO_3 in the determination of Cd, Cu, Pb, Zn and Fe in certified reference sediments and failed to recover the Fe with the procedure [6]. The use of HNO_3 in the determination mercury was found to achieve a <50% of recovery efficiency for organic form of mercury [7]. The poor recovery of single acids during digestion gave way to acid mixtures. Mineral acid mixtures were used not only for oxidation but also to convert metals into the chlorine or nitrate forms that are easily determined by the FAAS [5]. Adeloju recommended HNO_3: H_2SO_4 for the analysis of Hg in biological and environmental samples [7]. However, Tinggi *et al*. revealed that sulphuric acid in the matrix could significantly suppressed the absorbance signals especially for lead analysis thus reduce its sensitivity

Table 1: Metals content (mg/kg) with different acid digestion mixtures

Metals spiked / Digestion solutions	Fe 5.0mg/kg	Fe 10.0mg/kg	Zn 5.0mg/kg	Zn 10.0mg/kg	Pb 0.5mg/kg	Pb 1.0mg/kg
HNO_3	4.8 ± 1.1	8.8 ± 3.2	6.3 ± 0.4	11.5 ± 0.0	0.4 ± 0.2	1.5 ± 0.4
HNO_3: HCl (1:1)	6.5 ± 1.1	10.3 ± 0.4	6.0 ± 0.0	10.5 ± 0.0	0.4 ± 0.2	0.6 ± 0.2
HNO_3: HCl (1:2)	4.3 ± 1.1	9.3 ± 1.1	6.0 ± 0.7	8.3 ± 0.4	0.5 ± 0.1	1.0 ± 0.1
HNO_3: HCl (1:3)	4.5 ± 0.7	8.0 ± 0.7	4.3 ± 1.1	11.0 ± 0.0	0.5 ± 0.1	1.0 ± 0.1
HNO_3: H_2SO_4 (1:1)	3.5 ± 2.1	9.3 ± 1.1	4.0 ± 0.7	10.8 ± 0.4	0.9 ± 0.2	1.8 ± 0.4

Results and Discussion

Evaluation of acid digestion protocols

Table 1 shows the metals recovered with different acid digestion solutions. Statistically, no significant differences ($F_{crit} > F$) were reported at 95% confidence level for the determination of Fe and Zn with the selected acid digestion solutions. However, for Pb determination that appeared in trace amount, the results were significantly varied. The percentages recovery for the determination of Pb with HNO_3, HNO_3: HCl (1:1) and HNO_3: H_2SO_4 (1:1) [8]. In addition, is method, together with the single acid digestion protocol, was found to favour the formation of precipitate due to fatty residue. The organic residue could not be removed with concentrated acids, therefore ethanol was added for digestion at room temperature. Similar problem was identified with HNO_3: HCl (1:1) digestion. The aqua-regia (HNO_3: HCl, 1:3) was strongly recommended for digestion of diverse samples attributed to its complete digestion performance and good recoveries [5, 8]. In most cases, the recovery performance of HNO_3: HCl (1:2) corresponded well to the HNO_3: HCl (1:3) as illustrated in Fig. 1.

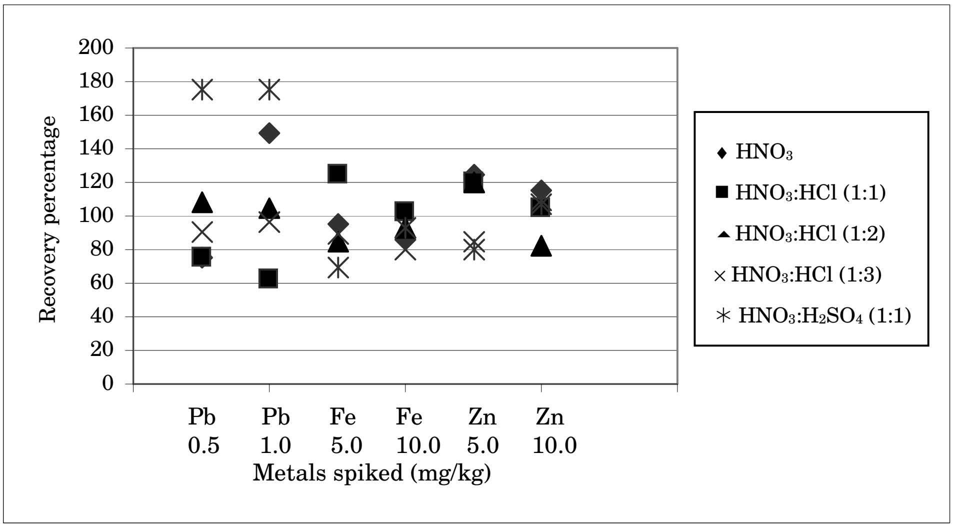

Figure 1: Recovery percentages of metals with different acid digestion solutions

The selected digestion solutions did not diverse greatly for metals that are present in considerable amount, as the percentages recovery obtained were rather consistent. However, for metals that exist at low concentration, the selection of an appropriate digestion protocol is crucial in order to obtain more reliable results. Concerning the reproducibility, the relative standard deviation values (R.S.D.) of the five digestion protocols were lower than 1% for determination of Fe and Zn. The R.S.D. values for Pb analysis were relatively high ranging from 2-9%; this was most likely due to the lower metal contents in the samples. The results obtained indicate that acid digestion with HNO_3: HCl in the ratio of 1:3 and 1:2 produced more reliable results for the analysis of Fe, Zn and Pb. In this study, digestion with HNO_3: HCl (1:2) was employed for the determination of Pb, Fe and Zn in canned sardines.

Table 2: Range of metals in canned sardines manufactured in Malaysia and Thailand

Manufacturing country	Pb (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Malaysia	0.55-1.35	61.20-166.35	24.42-57.06
Thailand	0.65-1.15	8.39-240.55	32.66-56.75
Permitted level	2	Not provided	100

Determination of Pb, Fe and Zn in canned sardines

According to the Food Act 1983 and the Food Regulations 1996 [10], the maximum permitted levels of Pb and Zn in fish products are 2 mg/kg and 100 mg/kg, respectively. Table 2 shows the range of metal contents in the canned sardines manufactured in Malaysia and Thailand. Apparently, the metal contents analyzed were well below the maximum permitted level, indicating that the products were safe for consumption. ANOVA was performed to compare if there is significant difference in the metal contents of the canned sardines manufactured from different countries. Statistically, no significant difference ($F_{crit} > F$) was observed at 95% confidence level for the Pb, Fe and Zn contents.

The average metal contents in canned sardines with different shelf lives ranging from one to three years are shown in Table 3. Statistical analysis at 95% confidence level with ANOVA demonstrated significant differences in Fe and Pb ($F_{crit} > F$). Apparently, Fe content increased significantly when canned sardines were kept for longer period of time. It was reported that the leaching of iron into the canned food is dependent on the storage time [11]. However, there is no statutory limit specified in the Food Act 1983 for the iron concentration in canned food. The concentration of Fe in canned food generally reflect that which occurs naturally and in some instances the concentration has been increased by corrosion of steel in the can. Iron is an essential trace element for biological activities. Some of the

Table 3 : Average metal contents in canned sardines with different shelf life

	Malaysia			Thailand		
Shelf life	Pb (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
< 1 year	0.7 ± 0.1	82.1 ± 18.6	34.0 ± 0.9	0.7 ± 0.0	39.3 ± 4.0	35.0 ± 2.1
1-2 years	1.1 ± 0.2	120.1 ± 34.9	46.1 ± 13.7	0.8 ± 0.2	146. 3 ± 24.6	47.8 ± 3.1
2-3 years	1.0 ± 0.1	137.0 ± 20.9	35.9 ± 4.9	0.9 ± 0.2	140.8 ± 14.1	38.8 ± 6.0

processed products are even fortified with iron content. It is evaluated as a deficiency problem rather than toxicological problem, therefore no health-based guideline is established. In spite of that, the daily intake recommended is 10-15 mg/day [11]. An increase was also observed for Pb content in relation to the shelf life of the canned sardines. The relationship of metal contents in canned food over the storage time is well established. Bigelow, and Adam and Horner showed that tin concentration in canned food increased over periods of a few months to two years [12,13]. This was subsequently supported by other investigators that canned food accumulated more tin and other metals when stored for longer periods [14,15]. Contamination of Pb in canned food may be attributed to two sources: the solder used in fabrication of can seams and the Pb contained in the tin coating on tinplate. The risk of lead contamination remains although technology of pure tin solder had been introduced as an alternative to lead solder. Higher material cost due to pure tin solder has hindered most of the canned industry from employing it. The level of Zn in canned sardines with varying shelf life was rather consistent indicating no contribution of Zn owing the canning process. The presence of Zn in the products was the characteristic of the natural occurrence.

the canned sardines. No Pb was detected with FAAS for all of the canned sardines manufactured in Malaysia and Thailand with shelf life of <1 year and 1-2 years. However, an average of 0.27 mg/kg of Pb was identified for products more than 2 years demonstrating possibility of Pb contamination from the cans. Elevated Fe content was also observed in the gravy from the canned sardines of different shelf life. The average Fe content of canned sardines with shelf life < 1 year, 1-2 years and 2-3 years were 5.74mg/kg, 8.55 mg/kg and 14.62 mg/kg, respectively.

The increase of Fe content revealed the possibility of Fe dissolution from cans into the food. The Zn content in the gravy however, varied in the range of 3.85 - 4.85 mg/kg throughout the shelf life. As a whole, canned sardines are likely to be subjected to Pb and Fe contamination from the can. Oduoza likewise reported greater concentrations of Sn, Fe and Pb in samples of tomato purees and orange juice from the shop compared to the immediate factory samples [16]. Canned food has been reported to consist of higher metal contents compared to other packaging approaches. Mesallam revealed relatively higher heavy metals contents in canned orange juice over those kept in paperboard box or laminate pouch [17].

Table 4 : Average metal contents in the gravy from canned sardines with different shelf life

	Malaysia			Thailand		
Shelf life	Pb (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
< 1 year	ND	5.6 ± 1.9	3.9 ± 0.4	ND	6.1 ± 1.1	3.9 ± 1.1
1-2 years	ND	7.3 ± 1.1	4.4 ± 0.3	ND	9.8 ± 2.4	4.2 ± 0.3
2-3 years	0.2 ± 0.0	13.9 ± 1.3	4.9± 1.3	0.3 ± 0.0	16.6 ± 1.3	4.9 ± 0.9

The gravy of the canned sardines was also analyzed for its Pb, Fe and Zn contents (Table 4). The results demonstrated a better indication on the relationship of shelf life with the metal contents in

Cans stored for longer periods are likely to be subjected to physical damage such as corrosion and dent. The iron in the mild steel base of tinplated or tin-free steel may dissolve into foods especially when the lacquer coating is damaged. Ten out of thirty cans of the canned sardines analysed were dented and corroded. The average Pb and Fe contents in the canned sardines with dented and rusted appearance were slightly elevated with 0.98mg/kg and 115.64 mg/kg, respectively. The Pb and Fe contents of the undented products however were 0.80 mg/kg and 102.97 mg/kg correspondingly. The results suggest that physical damage on the canned products implicates on the metals content especially those materials used in can-making industry. Nevertheless, statistical analysis revealed no significant difference at 95% confidence level for metal contents in cans with different appearances.

Conclusion

Evaluation of the acid digestion with different combination of solvents suggested that the acid digestion mixture of HNO_3: HCl in the ratio of 1:3 and 1:2 gave rise to reliable percentage recovery. Acid digestion mixture of HNO_3 and HCl (1:2) was employed for the determination of Fe, Pb and Zn in canned sardines. The metal concentrations were well below the level permitted, however, leaching of Fe and Pb from the can into the food was likely to have occurred for products stored for longer period of time.

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